Environmental and Molecular Mutagenesis 40:161–167 (2002)

Association of Prostate Cancer With Rapid *N*-acetyltransferase 1 (*NAT1*10*) in Combination With Slow *N*-acetyltransferase 2 Acetylator Genotypes in a Pilot Case-Control Study

David W. Hein,[1]* Matthew A. Leff,[1] Naoko Ishibe,[2] Rashmi Sinha,[3] Harold A. Frazier,[4] Mark A. Doll,[1] Gong H. Xiao,[1] Martin C. Weinrich,[5] and Neil E. Caporaso [2]

[1] *Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky*
[2] *Genetic Epidemiology Branch, National Cancer Institute, Bethesda, Maryland*
[3] *Nutritional Epidemiology Branch, National Cancer Institute, Bethesda, Maryland*
[4] *National Naval Medical Center, Bethesda, Maryland*
[5] *Department of Medicine, University of Louisville School of Medicine, Louisville, Kentucky*

N-acetyltransferase-1 (NAT1) and *N*-acetyltransferase-2 (NAT2) are important in the metabolism of aromatic and heterocyclic amine carcinogens that induce prostate tumors in the rat. We investigated the association of genetic polymorphisms in NAT1 and NAT2, alone and in combination, with human prostate cancer. Incident prostate cancer cases and controls in a hospital-based case-control study were frequency-matched for age, race, and referral pattern. The frequency of slow acetylator *NAT1* genotypes (*NAT1*14, *15, *17*) was 5.8% in controls but absent in cases. In contrast, in comparison with all other *NAT1* genotypes the putative rapid acetylator *NAT1* genotype (*NAT1*10*) was significantly higher in prostate cancer cases than controls (OR, 2.17; 95% CI, 1.08–4.33; $P = 0.03$). Combinations of *NAT1*10* with *NAT2* slow acetylator genotypes (OR, 5.08; 95% CI, 1.56–16.5; $P = 0.008$) or with *NAT2* very slow (homozygous *NAT2*5*) acetylator genotypes (OR, 7.50; 95% CI, 1.55–15.4; $P = 0.016$) further increased prostate cancer risk. The results of this small pilot study suggest increased susceptibility to prostate cancer for subjects with combinations of *NAT1*10* and slow (particularly very slow) *NAT2* acetylator genotypes. This finding should be investigated further in larger cohorts and in other ethnic populations. Environ. Mol. Mutagen. 40:161–167, 2002.

Key words: prostate cancer; *N*-acetyltransferase 1; *N*-acetyltransferase 2; acetylator genotype

INTRODUCTION

Both environmental and genetic factors are considered important in the etiology of human prostate cancer [Mettlin, 1997]. Some studies suggest a role for well-done meat [Norrish et al., 1999] and cigarette smoking [Hsing et al., 1991; Sharpe and Siemiztycki, 2001] in prostate cancer etiology, but the findings have been equivocal. Aromatic [Katayama et al., 1982; Ito et al., 1988; Shirai et al., 1990] and heterocyclic [Shirai et al., 1997, 1999] amine carcinogens induce prostate tumors in the rat. The *N*-hydroxy metabolites of these carcinogens exhibit increased carcinogenicity [Shirai et al., 1992; Archer et al., 2000]. Humans are exposed to these carcinogens in cigarette smoke [Manabe et al., 1991; Peluso et al., 1991] and well-cooked foods [Layton et al., 1995; Bogen and Keating, 2001]

Hepatic *N*-acetylation of aromatic amines competes with hepatic *N*-hydroxylation, whereas *O*-acetylation of the *N*-hydroxy metabolites in target tissues yields unstable acetoxy intermediates, resulting in electrophiles that initiate tumors [Hein et al., 2000a]. These acetylation reactions are catalyzed by *N*-acetyltransferase 1 (NAT1) and 2 (NAT2) and these enzymes exhibit genetic polymorphism in humans [Hein et al., 2000a].

Grant sponsor: the National Cancer Institute; Grant number: United States Public Health Service CA-34627.

Current address for H.A. Frazier: Department of Urology, George Washington University, Washington, DC.

*Correspondence to: David W. Hein, Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, KY 40292. E-mail: d.hein@louisville.edu

Received 9 April 2002; provisionally accepted 27 May 2002; and in final form 12 June 2002

DOI 10.1002/em.10103

Published online 25 September 2002 in Wiley InterScience (www.interscience.wiley.com).

Polymorphisms in *NAT1* yield over 25 variant alleles compared to the reference *NAT1*4* allele [Hein et al., 2000a]. A common *NAT1* allelic variant (*NAT1*10*) is associated with increased acetyltransferase catalytic activity (rapid acetylator phenotype) in vitro [Bell et al., 1995a] and in vivo [Hein et al., 2000b]. The *NAT1*10* acetylator phenotype is somewhat controversial because the increased catalytic activity has not been consistently reported in other studies [reviewed in Hein et al., 2000a]. Other less common *NAT1* allelic variants (*NAT1*14, *15, *17,* and **22*) are associated with reduced *N*- and *O*-acetyltransferase catalytic activities [Butcher et al., 1998; Lin et al., 1998; Fretland et al., 2001a, 2002; Hein, 2002].

Single nucleotide polymorphisms (SNPs) have been identified in *NAT2*, yielding over 25 variant alleles compared to the reference *NAT2*4* allele [Hein et al., 2000a]. Several of the SNPs result in alleles encoding for NAT2 proteins with reduced activity, but $G^{191}A$ ($R^{64}Q$) and $T^{341}C$ ($I^{114}T$) found in the *NAT2*5* and *NAT2*14* clusters result in the greatest reduction in NAT2 activity [Fretland at al., 2001b; Hein, 2002]. Slow acetylator phenotype (homozygous slow *NAT2* alleles) is associated with higher susceptibility to urinary bladder cancer upon exposure to aromatic amine carcinogens [Cartwright et al., 1982; Risch et al., 1995; Marcus et al., 2000]. In some studies, the association with urinary bladder cancer is greatest in the slowest NAT2 acetylator phenotypes [Cartwright et al., 1982] or those possessing *NAT2*5* genotypes [Brockmoller at al., 1996; Okkels et al., 1997; Filiadis et al., 1999].

If exposures to aromatic and/or heterocyclic arylamines are important in human prostate cancer etiology, individual risk should be modified by genetic polymorphism in enzymes that biotransform them. Since *N*-acetyltransferase 2 is expressed predominantly in liver, we hypothesized that individuals with the slow acetylator *NAT2* genotype (particularly the very slow *NAT2* acetylator genotype) would be at increased risk. The rationale for this hypothesis is that very slow NAT2 acetylators should have reduced hepatic *N*-acetylation of aromatic amine prostate carcinogens (detoxification) and therefore higher levels of hepatic *N*-hydroxylation (activation). Since NAT1 is expressed in prostate [Agundez et al., 1998; Lawson and Kolar, 2002], we further hypothesized that following transport of the *N*-hydroxylated metabolite(s) to the prostate, individuals with rapid *NAT1* acetylator genotype have greater activation (*O*-acetylation) and therefore higher risk of prostate cancer. Individuals with a combination of rapid *NAT1* and slow *NAT2* acetylator genotypes would be at greater risk for prostate cancer. We tested this hypothesis in a pilot hospital-based case-control study.

MATERIALS AND METHODS

Study Population

Fifty-seven men over the age of 50 with newly diagnosed, pathologically confirmed cancer of the prostate identified at the National Naval Medical Center (Bethesda, MD) were enrolled into the Genetics, Environment, and Metabolism Study from August 1993 to April 1995. Controls (127) were recruited from referrals from clinic physicians and from self-referrals generated by a recruitment flyer. Individuals with a previous diagnosis of malignancies (with the exception of skin cancer in situ) or other major medical illnesses were excluded. Hospital controls meeting the inclusion criteria were frequency-matched to the prostate cancer cases on age (±5 years), race, and referral pattern. Approximately 70% of the eligible subjects (57 of 82 eligible prostate cancer cases and 127 of 184 eligible controls) participated in the study. The primary reason for nonparticipation was subject refusal.

Data Collection

Information on potential risk factors, such as demographics, family history, smoking history, and other lifestyle variables were collected by trained interviewers and use of a self-administered questionnaire. Information on dietary intake and family history data was ascertained for each subject by self-administered questionnaires. Blood samples from the study participants were also collected at a second study visit. Upon collection, the samples were centrifuged and aliquoted into plasma, buffy coat, and red blood cell components. DNA was obtained from buffy coat by using a standard organic method for DNA extraction.

Genotype Determinations

NAT1 genotype was determined using a polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) assay as previously described [O'Neil et al., 2000]. The assay is designed to distinguish over 20 human *NAT1* alleles (*NAT1*3, *4, *5, *10, *11, *14A, *14B, *15, *16, *17, *18A, *18B, *19, *20, *21 *22, *24, *25, *26A, *26B,* and **28*).

NAT2 genotype was determined using a PCR-RFLP assay previously described [Deitz et al., 2000] that is designed to distinguish over 25 human *NAT2* alleles (*NAT2*4, *5A,B,C,D,E,F, *6A,B,C,D,E *7A,B, *11, *12A,B,C, *13, *14A,B,C,D,E,F,G, *17, *18*).

All analyses were conducted with laboratory personnel blinded to case status. Positive and negative controls were used to ensure the activity of enzymes and reagents and to ensure that samples were not contaminated with foreign DNA. Any ambiguous result was repeated and/or confirmed by automated sequencing. Random samples were confirmed for genotype determination using a Taqman allele discrimination method for determination of *NAT1* [Doll and Hein, 2002] or *NAT2* [Doll and Hein, 2001] genotype.

Statistical Analyses

Crude odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by Fisher's Exact Test. Chi-square tests were used to test the statistical significance of trends involving more than two categories. All *P* values were based on two-sided hypothesis tests. The absence of cases with slow *NAT1* genotype required a substitution of 0.5 for 0 in order to generate the OR. Subjects were frequency-matched to assure similar numbers of subjects in major age strata. Residual small differences in the age distribution were corrected using an age and race term in the logistic regression model. Results were adjusted for race, age, body mass index (BMI), smoking history, and/or family history of prostate cancer. Since these adjustments did not change the results, the unadjusted findings are presented. Since the number of slow acetylator *NAT1* genotypes was very low, all nonrapid *NAT1* genotypes were combined to serve as the reference group. Similarly, because of the small number of homozygous rapid *NAT2* acetylators, homozygous rapid and heterozygous *NAT2* acetylators (possessing *NAT2*4, *12* or **13*) were combined to serve as the reference group. Since there were only nine subjects who were current smokers, current and past smokers were collapsed into a single group.

TABLE I. Descriptive Statistics of Study Population

Variable	Controls (%)	Cases (%)	*P* value
Age (years)	68.9 ± 7.3	69.0 ± 7.1	0.91
Body Mass Index	21.8 ± 3.3	21.8 ± 2.9	0.99
Race (% Caucasian)	94.4%	90.6%	
Education (years)[a]			
1–8	1 (0.8%)	1 (1.9%)	
9–12	10 (8.0%)	7 (13.2%)	
College	42 (33.6%)	16 (30.2%)	
Graduate	72 (57.6%)	29 (54.7%)	0.65
Smoking History[a]			
Never	44 (35.2%)	17 (32.1%)	
Ever	74 (59.2%)	34 (64.8%)	
Current	7 (5.6%)	2 (3.8%)	0.78
Pack-years[b]	28.3 ± 25.1	23.9 ± 22.7	0.36

[a]Information on education and smoking history not available for two controls and four cases.
[b]Based on smokers only (74 controls and 34 cases).

TABLE II. *NAT1* Alleles and Prostate Cancer

NAT1 allele	Controls (%)	Cases (%)
3	6 (2.5)	0 (0)
4	183 (75.6)	70 (74.5)
10	42 (17.4)	22 (23.4)
11	4 (1.7)	2 (2.1)
14	5 (2.1)	0 (0)
15	1 (0.4)	0 (0)
17	1 (0.4)	0 (0)
Total	242 (100)	94 (100)

RESULTS

Descriptive information was obtained from 53/57 cases and 125/127 controls (Table I). Over 90% of the cohort was Caucasian with a mean age of about 69 years at the time of the study. Over 50% of both cases and controls had a smoking history. The cases and controls did not differ significantly with respect to age, BMI, race, education, or smoking history (Table I).

The frequencies of *NAT1* alleles and genotypes in cases and controls are shown in Tables II and III. The *NAT1*4* allele and the *NAT1*4/*4* genotype were most common in this Caucasian population, followed by *NAT1*10* allele and *NAT1*4/*10* genotype. As shown in Table II, the *NAT1*14, *15, *17* alleles, associated with slow acetylator phenotype, were observed only among the controls. In contrast, the frequency of the *NAT1*10* allele was higher in cases (23.4%) than controls (17.4%). As shown in Table III and summarized in Table IV, the rapid *NAT1* genotype (*NAT1*10*) was overrepresented in prostate cancer (OR, 2.17; 95% CI, 1.08–4.33). This effect of *NAT1*10* genotype on prostate cancer risk was observed in ever smokers (OR, 2.59; 95% CI, 1.13–5.96) but not in never smokers (OR, 0.98; 95% CI, 0.26–3.71).

The frequency of *NAT2* alleles in cases and controls is shown in Table V. The most frequent *NAT2* alleles in both cases and controls were *NAT2*5B* > *NAT2 6A* > *NAT2*4*. The frequency of rapid *NAT2* acetylator alleles was higher in controls (25.2%) than cases (17.0%), whereas the frequency of very slow acetylator *NAT2* alleles (*NAT2*5* and *NAT2*14*) was lower in controls (42.2%) than cases (50.0%). The frequency of *NAT2* genotypes in cases and controls is shown in Table VI. The slow *NAT2* acetylator genotype was not significantly associated with prostate cancer (OR, 1.78; 95% CI, 0.88–3.60). The association between individuals homozygous for *NAT2*5* alleles (possessing $T^{341}C$) and prostate cancer (OR, 2.43; 95% CI, 0.96–6.12) approached significance ($P = 0.08$). The slow *NAT2* acetylator genotype was not associated with prostate cancer when stratified for smoking (data not shown).

Based on the considerations indicated earlier, if we postulate that rapid *NAT1* and slow *NAT2* are risk factors and

TABLE III. *NAT1* Genotype and Prostate Cancer

NAT1 genotype	Controls (%)	Cases (%)
3/4	4 (3.3)	0
3/10	1 (0.8)	0
3/11	1 (0.8)	0
4/4	73 (60.3)	23 (48.9)
4/10	26 (21.5)	22 (46.8)
4/11	1 (0.8)	2 (4.3)
4/14	5 (4.5)	0
4/17	1 (0.8)	0
10/10	7 (5.8)	0
10/15	1 (0.8)	0
11/11	1 (0.8)	0
Total	121 (100)	47 (100)

TABLE IV. *NAT1* Genotype and Prostate Cancer

NAT1 genotype	Controls (%)	Cases (%)	Crude OR (95% CI)
Not rapid (not *NAT1*10*)	86 (71.1)	25 (53.2)	1.0 (ref.)
Rapid (*NAT1*10*)	35 (28.9)	22 (46.8)	2.17 (1.08–4.33) $P = 0.03$

TABLE V. *NAT2* Allelic Frequencies in Prostate Cancer

NAT2 Allele	Controls (%)	Cases (%)
4	53 (23.0)	15 (16.0)
12A	2 (0.9)	1 (1.1)
13	3 (1.3)	0
Subtotal Rapid	58 (25.2)	16 (17.0)
6A	72 (31.3)	30 (31.9)
7B	3 (1.3)	1 (1.1)
Subtotal, **6, *7*	75 (32.6)	31 (33.0)
5A	5 (2.2)	0
5B	90 (39.1)	44 (46.8)
5C	2 (0.9)	2 (2.1)
14B	0	1 (1.1)
Subtotal **5, *14*	97 (42.2)	47 (50.0)

TABLE VI. *NAT2* Genotypes in Prostate Cancer

NAT2 genotype	Controls (%)	Cases (%)	Crude OR (95% CI)
4/4	3 (2.6)	0	
4/5B	23 (19.8)	7 (14.9)	
4/5C	0	1 (2.1)	
4/6A	22 (19.0)	7 (14.9)	
4/7B	2 (1.7)	0	
5B/13	2 (1.7)	0	
5B/12A	2 (1.7)	0	
6A/13	1 (0.9)	0	
12A/14B	0	1 (2.1)	
Total Rapid	55 (47.8)	16 (34.0)	1.0 (ref.)
5A/5B	2 (1.7)	0	
5A/6A	3 (2.6)	0	
5B/5B	14 (12.1)	12 (25.5)	
5B/5C	1 (0.9)	0	
5B/6A	31 (26.7)	12 (25.5)	
5B/7B	1 (0.9)	1 (2.1)	
5C/6A	1 (0.9)	1 (2.1)	
6A/6A	7 (6.0)	5 (10.6)	
Total Slow	60 (52.2)	31 (66.0)	1.78 (0.88–3.60) $P = 0.12$
Subtotal **5/*5*	17 (14.8)	12 (25.5)	2.43 (0.96–6.12) $P = 0.08$

TABLE VII. Combination *NAT2/NAT1*10* Genotypes in Prostate Cancer

NAT2/NAT1 at risk genotypes[a]	Controls (%)	Cases (%)	Crude OR (95% CI)[a]
0	36 (31.3)	6 (13.3)	1.0 (Ref.)
1	66 (57.4)	28 (62.2)	2.55 (0.96–6.72) $P = 0.058$
2	13 (11.3)	11 (24.4)	5.08 (1.56–16.5) $P = 0.008$
2[b]	4 (3.5)	5 (11.1)	7.50 (1.55–15.4) $P = 0.016$

[a]At risk genotypes defined as *NAT2* slow and *NAT1* rapid acetylator (*NAT1**10) genotypes.
[b]*NAT2* slow acetylator genotypes restricted to homozygous *NAT2*5*.

evaluate risk based on the total number of "at risk" alleles observed, prostate cancer risk was 5-fold higher (OR, 5.08; 95% CI, 1.56–16.5; $P = 0.008$) for rapid *NAT1* in combination with the slow *NAT2* acetylator genotypes and over 7-fold higher (OR, 7.50; 95% CI, 1.55–15.4; $P = 0.016$) for rapid *NAT1* in combination with the very slow (*NAT2*5*) acetylator genotype (Table VII).

DISCUSSION

The rapid *NAT1* genotype (*NAT1*10*) was associated with prostate cancer in this small pilot case-control study (OR, 2.17; 95% CI, 1.08–4.33; $P = 0.03$). This result is similar to a previous report that found a significant association (OR, 2.4; 95% CI, 1.0–5.6) between *NAT1*10* and prostate cancer in a Japanese population [Fukutome et al., 1999]. In addition to prostate cancer [Fukutome et al., 1999], associations of the *NAT1*10* genotype with breast [Millikan et al., 1998; Krajinovic et al., 2001], colorectal [Bell et al., 1995b; Chen et al., 1998; Ishibe et al., 2002], urinary bladder [Taylor et al., 1998; Katoh et al., 1999; Cascorbi et al., 2001], oral [Katoh et al., 1998], lung [Wikman et al., 2001], larynx [Varzim et al., 2002], and gastric [Boissy et al., 2000] cancers have been reported. However, other studies have not found an association between the *NAT1*10* genotype and colorectal [Katoh et al., 2000; Butler et. al., 2001; Houston and Tomlinson, 2001], urinary bladder [Vaziri et al., 2001], head and neck [Olshan et al., 2001; Fronhoffs et al., 2001], hepatitis B-related hepatocarcinoma [Yu et al., 2000], and childhood acute lymphoblastic anemia [Krajinovic et al., 2000]. The *NAT1*10* phenotype and its association with cancer susceptibility remain poorly understood [Brockton et al., 2000; Hein et al., 2000a; Hein, 2002].

The slow *NAT2* acetylator genotype was not significantly associated with prostate cancer in this study (OR, 1.75; 95% CI, 0.86–3.54). However, the association between individuals homozygous for *NAT2*5* alleles (possessing $T^{341}C$) and prostate cancer (OR, 2.43; 95% CI, 0.96–6.12) approached significance ($P = 0.08$). Recent studies investigating the relationship of *NAT2* acetylator genotype and prostate cancer also did not find a significant association between *NAT2* acetylator genotype and prostate cancer [Agundez et al., 1998; Wadelius et al., 1999]. These studies did not test the effect of *NAT2*5* separately.

Consistent with our hypothesis, prostate cancer risk was 5-fold higher (OR, 5.08; 95% CI, 1.56–16.5; $P = 0.008$) for rapid *NAT1* in combination with the slow *NAT2* acetylator genotypes and over 7-fold higher (OR, 7.50; 95% CI, 1.55–15.4; $P = 0.016$) in combination with the very slow (*NAT2*5*) acetylator genotypes. The fact that *NAT1*10* is in linkage disequilibrium with the rapid *NAT2*4* acetylator genotype [Smelt et al., 1998; Henning et al., 1999; Cascorbi et al., 2001] may tend to mask the association of *NAT1*10* genotype with prostate cancer. Thus, we would expect the effect of *NAT1*10* on prostate cancer risk to be greater when not linked to rapid (i.e., *NAT2*4*) acetylator genotype. Our findings suggest that *N*-acetylation via hepatic NAT2 is important in the detoxification of prostate carcinogens, whereas *O*-acetylation catalyzed by NAT1 expressed in prostate epithelial cells is important for their activation. Recent studies have measured *N*-acetyltransferase mRNA [Wang et al., 1999; Cui et al., 2000] and catalytic activity [Agundez et al., 1998; Yeh et al., 2000, 2001; Lawson and Kolar, 2002] in human prostate. Two of the studies [Agundez et al., 1998; Lawson and Kolar, 2002] clearly showed that human prostate *N*-acetyltransferase activity was catalyzed by NAT1 and not NAT2. Human prostate epithelial cells have been shown to activate heterocyclic amines [Williams et al., 2000; Lawson and Kolar, 2002] and *N*-hydroxy heterocyclic amines [Wang et al., 1999; Williams et al., 2000] to DNA adducts, which is consistent with the activation role for NAT1 within the prostate.

Previous studies have shown that the combination of the rapid *NAT1* acetylator genotype (*NAT1*10*) with the slow *NAT2* acetylator genotype yielded the highest level of DNA adducts in human urinary bladder [Badawi et al., 1995] and the highest frequency of urinary bladder cancers [Taylor et al., 1998]. A similar finding has been reported recently for lung cancer [Wikman et al., 2001]. This is the first report of this finding for human prostate cancer, but the analogy is the same (i.e., one would expect the highest DNA adduct levels and prostate cancer frequency for the combination of rapid *NAT1* and slow *NAT2* acetylator genotypes). Thus, our pilot study suggests that the *NAT1* and *NAT2* acetylation polymorphisms both modify prostate cancer risk slightly, but in combination the increased risk is additive. It is likely that polymorphisms in other carcinogen-metabolizing enzymes also increase risk. The results of this small pilot study are consistent with our hypothesis but need to be confirmed in larger sample sizes and in other ethnic groups.

ACKNOWLEDGMENTS

The views expressed in this article are those of the authors and do not reflect the official policy of the United States Navy, Department of Defense, or the US Government. We thank John Berlot, James Frame, Margaret Tucker, Richard Hayes, Robert Hoover, Keven O'Connell, and Jane Cowan for assistance in the design and conduct of this study. Portions of this work were presented at the Society of Toxicology meeting held in Nashville, TN (March, 2002) and constituted partial fulfillment by Matthew A. Leff for the Ph.D. in pharmacology and toxicology at the University of Louisville.

REFERENCES

Agundez JA, Martinez C, Olivera M, Gallardo L, Ladero JM, Rosad C, Prados J, Rodriguez-Molina J, Resel L, Benitez J. 1998. Expression in human prostate of drug-and carcinogen-metabolizing enzymes: association with prostate cancer risk. Br J Cancer 78:1361–1367.

Archer L, Morse P, Jones RF, Shirai T, Haas GP, Wang CY. 2000 Carcinogenicity of the N-hydroxy derivative of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline and 3,2'-dimethyl-4-aminobiphenyl in the rat. Cancer Lett 155:55–60.

Badawi AF, Hirvonen A, Bell DA, Lang NP, Kadlubar FF. 1995. Role of aromatic amine acetyltransferases, NAT1 and NAT2, in carcinogen-DNA adduct formation in the urinary bladder. Cancer Res 55:5230–5237.

Bell DA, Badawi AF, Lang NP, Ilett KF, Kadlubar F, Hirvonen A. 1995a. Polymorphism in the NAT1 polyadenylation signal: association of NAT1*10 allele with higher N-acetylation activity in bladder and colon tissue samples. Cancer Res 55:5226–5229.

Bell DA, Stephens E, Castranio T, Umbach DM, Watson M, Deakin M, Elder J, Duncan H, Hendrickse C, Strange RC. 1995b. Polyadenylation polymorphism in the N-acetyltransferase gene 1 (NAT1) increases risk for colorectal cancer. Cancer Res 55:3537–3542.

Bogen KT, Keating GA. 2001. U.S. dietary exposures to heterocyclic amines. J Expo Anal Environ Epidemiol 11:155–168.

Boissy RJ, Watson MA, Umbach DM, Deakin M, Elder J, Strange RC, Bell DA. 2000. A pilot study investigating the role of NAT1 and NAT2 polymorphisms in gastric adenocarcinoma. Int J Cancer 87:507–511.

Brockmoller J, Cascorbi I, Kerb R, Roots I. 1996. Combined analysis of inherited polymorphisms in arylamine N-acetyltransferase 2, glutathione S-transferases M1 and T1, microsomal epoxide hydrolase, and cytochrome P450 enzymes as modulators of bladder cancer risk. Cancer Res 56:3915–3925.

Brockton N, Little J, Sharp L, Cotton SC. 2000. N-acetyltransferase polymorphisms and colorectal cancer: a HuGE review. Am J Epidemiol 151:846–861.

Butcher NJ, Ilett KF, Minchin RF. 1998. Functional polymorphism of the human arylamine N-acetyltransferase type 1 gene caused by C190T and G590A mutations. Pharmacogenetics 8:67–72.

Butler WJ, Ryan P, Roberts-Thomson IC. 2001. Metabolic genotypes and risk for colorectal cancer. J Gastroenterol Hepatol 16:631–635.

Cartwright RA, Glashan RW, Rogers HJ, Ahmad RA, Barham-Hall D, Higgens E, Kahn M. 1982. A role of N-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. Lancet 2:842–846.

Cascorbi I, Roots I, Brockmoller J. 2001. Association of NAT1 and NAT2 polymorphisms to urinary bladder cancer: significantly reduced risk in subjects with NAT1*10. Cancer Res 61:5051–5056.

Chen J, Stampfer MJ, Hough HL, Garcia-Closas M, Willet WC, Hennekens, CH, Kelsey KT, Hunter D. 1998. A prospective study of N-acetyltransferase genotype, red meat intake, and risk of colorectal cancer. Cancer Res 58:3307–3311.

Cui L, Takahashi S, Tada M, Kato K, Yamada Y, Kohri K, Shirai T. 2000. Immunohistochemical detection of carcinogen-DNA adducts in normal human prostate tissues transplanted into the subcutis of athymic nude mice: results with 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 3,2'-dimethyl-4-aminobiphenyl (DMAB) and relation to cytochrome P450s and N-acetyltransferase activity. Jpn J Cancer Res 91:52–58.

Deitz AC, Zheng W, Leff MA, Gross M, Wen W-Q, Doll MA, Xiao GH, Folsom AR, Hein DW. 2000. N-acetyltransferase-2 genetic polymorphism, well-done meat intake, and breast cancer risk among postmenopausal women. Cancer Epidemiol Biomark Prev 9:905–910.

Doll MA, Hein DW. 2001. Comprehensive human NAT2 genotype method using single nucleotide polymorphism-specific polymerase chain reaction primers and fluorogenic probes. Anal Biochem 288:106–108.

Doll MA, Hein DW. 2002. Rapid genotype method to distinguish frequent and/or functional polymorphisms in human N-acetyltransferase-1 (NAT1). Anal Biochem 301:328–332.

Filiadis IF, Georgiou I, Alamanos Y, Kranas V, Giannakopoulos X, Lolis D. 1999 Genotypes of N-acetyltransferase-2 and risk of bladder cancer: a case-control study. J Urol 161:1672–1675.

Fretland AJ, Doll MA, Leff MA, Hein DW. 2001a. Functional characterization of nucleotide polymorphisms in the coding region of human N-acetyltransferase 1. Pharmacogenetics 11:511–520.

Fretland AJ, Leff MA, Doll MA, Hein DW. 2001b. Functional characterization of human N-acetyltransferase 2 (NAT2) single nucleotide polymorphisms. Pharmacogenetics 11:207–215.

Fretland AJ, Doll MA, Zhu Y, Smith L, Leff MA, Hein DW. 2002. Effect of nucleic acid substitutions in N-acetyltransferase-1 on N-acetylation (deactivation) and O-acetylation (activation) of arylamine carcinogens: implications for cancer predisposition. Cancer Detect Prev 26:10–14.

Fronhoffs S, Bruning T, Ortiz-Pallardo E, Brode P, Loch B, Harth V, Sachinidis, A, Bolt HM, Herberhold C, Vetter H, Ko, Y. 2001. Real-time PCR analysis of the N-acetyltransferase NAT1 allele *3, *4, *10, *11, *14, and *17 polymorphisms in squamous cell cancer of head and neck. Carcinogenesis 22:1405–1412.

Fukutome K, Watanabe M, Shiraishi T, Murata M, Uemura H, Kubota Y, Kawamura J, Ito H, Yatani R. 1999. N-acetyltransferase 1 genetic polymorphism influences the risk of prostate cancer development. Cancer Lett 136:83–87.

Hein DW. 2002. Molecular genetics and function of NAT1 and NAT2: Role in aromatic amine metabolism and carcinogenesis. Mutat Res 506–507: 65–77.

Hein DW, Doll MA, Rustan TD, Gray K, Feng Y, Ferguson RJ, Grant DM. 1993. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. Carcinogenesis 14:1633–1638.

Hein DW, Doll MA, Rustan TD, Ferguson RJ. 1995. Metabolic activation of N-hydroxyarylamines and N-hydroxyarylamides by 16 recombinant human NAT2 allozymes: effects of 7 specific NAT2 nucleic acid substitutions. Cancer Res 55:3531–3536.

Hein DW, Doll MA, Fretland AJ, Leff MA, Webb SJ, Xiao GH, Devanaboyina U-S, Nangju NA, Feng Y. 2000a. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. Cancer Epidemiol Biomark Prev 9:29–42.

Hein DW, McQueen CA, Grant DM, Goodfellow GH, Kadlubar FF, Weber WW. 2000b. Pharmacogenetics of the arylamine N-acetyltransferases: a symposium in honor of Wendell W. Weber. Drug Metab Dispos 28:1425–1432.

Henning S, Cascorbi I, Munchow B, Jahnke V, Roots I. 1999. Association of arylamine N-acetyltransferases NAT1 and NAT2 genotypes to laryngeal cancer risk. Pharmacogenetics 9:103–111.

Houston RS, Tomlinson IP. 2001. Polymorphisms and colorectal tumor risk. Gastroenterology 121:282–301.

Hsing AW, McLaughlin JK, Hrubec A, Blot WJ, Fraumeni FJ Jr. 1991. Tobacco use and prostate cancer: 26-year follow-up of US veterans. Am J Epidemiol 133:437–441.

Ishibe N, Sinha R, Hein DW, Kulldorff M, Strickland P, Fretland AJ, Chow W-H, Kadlubar FF, Lang NP, Rothman N. 2002. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. Pharmacogenetics 12:145–150.

Ito N, Shirai T, Tagawa Y, Nagamura A, Fukushima S. 1988. Variation in tumor yield in the prostate and other target organs of the rat in response to varied dosage and duration of administration of 3,2-dimethyl-4-aminobiphenyl. Cancer Res 48:4629–4632.

Katayama S, Fiala E, Reddy BS, Rivenson A, Silverman J, Williams GM, Weisburger JH. 1982. Prostate adenocarcinoma in rats: Induction by 3,2-dimethyl-4-aminobiphenyl. J Natl Cancer Inst 68:867–873.

Katoh T, Kaneko S, Boissy R, Watson M, Ikemura K, Bell DA. 1998. A pilot study testing the association between N-acetyltransferases 1 and 2 and risk of oral squamous cell carcinoma in Japanese people. Carcinogenesis 19:1803–1807.

Katoh T, Inatomi H, Yang M, Kawamato T, Matsumota T, Bell DA. 1999. Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) genes and risk of urothelial transitional cell carcinoma among Japanese. Pharmacogenetics 9:401–404.

Katoh T, Boissy R, Nagata N, Kitagawa K, Kuroda Y, Itoh H, Kawamoto T, Bell DA. 2000. Inherited polymorphism in the N-acetyltransferase 1 (NAT1) and 2 (NAT2) genes and susceptibility to gastric and colorectal adenocarcinoma. Int J Cancer 85:46–49.

Krajinovic M, Richer C, Sinnett H, Labuda D, Sinnett D. 2000. Genetic polymorphisms of N-acetyltransferases 1 and 2 and gene-gene interaction in the susceptibility to childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomark Prev 9:557–562.

Krajinovic M, Ghadirian P, Richer C, Sinnett H, Gandini S, Perret C, Lacroix A, Labuda D, Sinnett D. 2001. Genetic susceptibility to breast cancer in French-Canadians: role of carcinogen-metabolizing enzymes and gene-environment interactions. Int J Cancer 92:220–225.

Lawson T, Kolar C. 2002. Human prostate epithelial cells metabolize chemicals of dietary origin to mutagens. Cancer Lett 175:141–146.

Layton DW, Bogen KT, Knize MG, Hatch FT, Johnson VM, Felton JS. 1995. Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. Carcinogenesis 16:39–52.

Lin HJ, Probst-Hensch NM, Hughes NC, Sakamoto GT, Louis AD, Kau JH, Lin BK, Lee DB, Lin J, Frankl HD, Lee ER, Hardy S, Grant DM, Haile RW. 1998. Variants of N-acetyltransferase NAT1 and a case-control study of colorectal adenomas. Pharmacogenetics 8:269–281.

Manabe S, Tohyama K, Wada O, Aramaki T. 1991. Detection of a carcinogen, 2-amino-1-methyl-6-imidazo[4,5-b]pyridine (PhIP) in cigarette smoke condensate. Carcinogenesis 12:1945–1947.

Marcus PM, Hayes RB, Vineis P, Garcia-Closas M, Caporaso NE, Autrup H, Branch RA, Brockmoller J, Ishizaki T, Karakaya AE, Ladero JM, Mommsen S, Okkels H, Romkes M, Roots I, Rothman N. 2000. Cigarette smoking, N-acetyltransferase 2 acetylation status, and bladder cancer risk: a case-series meta-analysis of a gene-environment interaction. Cancer Epidemiol Biomarker Prev 9:461–467.

Mettlin C. 1997. Recent developments in the epidemiology of prostate cancer. Eur J Cancer Prev 33:340–347.

Millikan RC, Pittman GS, Newman B, Tse C-K, Selmin O, Rockhill B, Savitz D, Moorman PG, Bell DA. 1997. Cigarette smoking, N-acetyltransferases 1 and 2 and breast cancer risk. Carcinogenesis 18:2127–2132.

Norrish AE, Ferguson LR, Knize MG, Felton JS, Sharpe SJ, Jackson RT. 1999. Heterocyclic amine content of cooked meat and risk of prostate cancer. J Natl Cancer Inst 91:2038–2044.

Okkels H, Sigsgaard T, Wolf H, Autrup H. 1997. Arylamine N-acetyltransferase 1 (NAT1) and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. Cancer Epidemiol Biomarker Prev 6:225–231.

Olshan AF, Weissler MC, Watson MA, Bell DA. 2000. GSTM1, GSTT1, GSTP1, CYP1A1 and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. Cancer Epidemiol Biomark Prev 9:185–191.

O'Neil WM, Drobitch RK, MacArthur RD, Farrough MF, Doll MA, Fretland AJ, Hein DW, Crane LR, Svensson CR. 2000. Acetylator phenotype and genotype in patients infected with HIV: discordance between methods for phenotype determination and genotype. Pharmacogenetics 10:171–182.

Peluso M, Castegnaro M, Malaveille C, Friesen M, Garren L, Hautefeuille A, Vineis P, Kadlubar FF, Bartsch H. 1991. ^{32}P postlabelling analysis of urinary mutagens from smokers of black tobacco implicates 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) as a major DNA-damaging agent. Carcinogenesis 12:713–717.

Risch A, Wallace DM, Bathers S, Sim E. 1995. Slow N-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. Hum Mol Genet 4:231–236.

Sharpe CR, Siemiatycki J. 2001. Joint effects of smoking and body mass index on prostate cancer risk. Epidemiology 12:546–551.

Shirai T, Nakamura A, Fukushima S, Yamamoto A, Tada M, Ito N. 1990. Different carcinogenic responses in a variety of organs, including the prostate, of five different rats strains given 3,2'-dimethyl-4-aminobiphenyl. Carcinogenesis 11:793–797.

Shirai T, Iwaski S, Naito H, Masui T, Kato T, Imaida K. 1992. Dose dependence of N-hydroxy-3,2'-dimethyl-4-aminobiphenyl-induced rat prostate carcinogenesis. Jpn J Cancer Res 83:695–698.

Shirai T, Sano M, Tamano S, Takahasi S, Hirose M, Futauchi M, Hasegawa R, Imaida K, Matsumoto K, Wakabayashi K, Sugimura T, Ito N. 1997. The prostate: a target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4-5,b]pyridine (PhIP) derived from cooked foods. Cancer Res 57:195–198.

Shirai T, Cui L, Takahashi S, Futakuchi M, Asamoto M, Kato R, Ito N. 1999. Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat prostate and induction of invasive

carcinomas by subsequent treatment with testosterone propionate. Cancer Lett 143:217–221.

Smelt VA, Mardon HJ, Sim E. 1998. Placental expression of arylamine N-acetyltransferases: evidence for linkage disequilibrium between NAT1*10 and NAT2*4 alleles of the two human N-acetyltransferase loci NAT1 and NAT2. Pharmacol Toxicol 83:149–157.

Taylor JA, Umbach DM, Stephens E, Castranio T, Paulson D, Roberson C, Johler, JL, Bell DA. 1998. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene exposure three-way interaction. Cancer Res 58:3603–3610.

Varzim G, Monteiro E, Silva R, Pinheiro C, Lopes C. 2002. Polymorphisms of the arylamine N-acetyltransferase (NAT1 and NAT2) and larynx cancer susceptibility. J Otorhinolaryngol Relat Spec 64:206–212.

Vaziri SA, Hughes NC, Sampson H, Darlington G, Jewett MA, Grant DM. 2001. Variation in enzymes of arylamine procarcinogen biotransformation among bladder cancer patients and control subjects. Pharmacogenetics 11:7–20.

Wadelius M, Autrup JL, Stubbins MJ, Andersson S-O, Johansson J-E, Wadelius C, Wolf CR, Autrup H, Rane A. 1999. Polymorphisms in NAT2, CYP2D6, CYP2C19, and GSTP1 and their association with prostate cancer. Pharmacogenetics 9:333–340.

Wang CY, Debiec-Rychter M, Schut HA, Morse P, Jones RJ, Archer C, King CM, Haas GP. 1999. N-acetyltransferase expression and DNA binding of N-hydroxyheterocyclic amines in human prostate epithelium. Carcinogenesis 20:1591–1595.

Wikman H, Thiel S, Jager B, Schmezer P, Spiegelhalder B, Edler L, Dienemann H, Kayser K, Schulta V, Drings P, Bartsch H, Risch A. 2001. Relevance of N-acetyltransferase 1 and 2 (NAT1, NAT2) genetic polymorphisms in non-small cell lung cancer susceptibility. Pharmacogenetics 11:157–168.

Williams JA, Martin FL, Muir GH, Hewer A, Grover PL, Phillips DH. 2000. Metabolic activation of carcinogens and expression of various cytochromes P450 in human prostate tissue. Carcinogenesis 21:1683–1689.

Yeh CC, Chung JG, Wu HC, Li YC, Lee M, Hung CF. 2000. Effects of butylated hydroxyanisole and butylated hydroxytoluene on DNA adduct formation and arylamines N-acetyltransferase activity in PC-3 cells (human prostate tumor) in vitro. Food Chem Toxicol 38:977–983.

Yeh CC, Hung CF, Wang WL, Chung JG. 2001. Kinetics of acetyl coenzyme A: arylamine N-acetyltransferase from rapid and slow acetylator human benign prostatic hyperplasia tissues. Urol Res 29:311–316.

Yu MW, Pai CI, Yang SY, Hsiao TJ, Chang HC, Lin SM, Liaw YF, Chen PJ, Chen CJ. 2000. Role of N-acetyltransferase polymorphisms in hepatitis B related hepatocellular carcinoma: impact of smoking on risk. Gut 47:703–709.

Accepted by—
F.F. Kadlubar